

FINAL REPORT

**ANTIBODY CONJUGATES FOR SEROLOGICAL DIAGNOSES
OF SPECIALIZED LIVESTOCK**

19970220

February 2000

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Canada¹

Saskatchewan

AGRI-FOOD INNOVATION FUND (AFIF)
PROJECT 97000220
RES-ID 90-FE

Final Report, February 1, 2000

**Submitted by the Centre for Animal Parasitology, Health of Animals
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Saskatoon.**

**ANTIBODY CONJUGATES FOR SEROLOGICAL DIAGNOSES OF DISEASES OF
SPECIALIZED LIVESTOCK.**

SUMMARY

Antibody conjugates are necessary reagents for tests like enzyme-linked immunosorbent assay (ELISA) or Western blot used in the serological diagnoses of animal diseases. Bison, caribou, and mule deer conjugates were prepared by chemically linking rabbit antibodies (i.e., immunoglobulin G or IgG) that react with bison-, caribou-, or mule deer-IgG, with an enzyme, alkaline phosphatase or horse-radish peroxidase. Six antibody conjugates were produced and their activities are similar to, or up to eight-fold greater than that of a commercially available rabbit anti-white tailed deer IgG. The reagents are adequate to perform ELISA tests on over 25,000 animals of each species.

TECHNICAL REPORT

Background and Objectives

Early and reliable diagnoses of infections of specialized livestock species can be achieved by serological diagnostic testing, which relies on the response of the immune system of an infected animal to the infectious agent. Upon infection, an animal develops an immune response which is characterized by the production of antibodies of different classes of which Immunoglobulin G (IgG) is predominant. IgG that binds specifically to a particular infectious agent begins to appear in the host 7 - 14 days after exposure and persists throughout the course of the disease. The specific IgG in an infected animal can be detected in a three-step procedure in the laboratory. First, the suspected infectious agent or an antigen derived from it is immobilized on a solid support (e.g., plastic). Second, serum from an infected animal, containing the specific IgG, is allowed to bind to the infectious agent or the antigen derived from it. Finally, the binding of the

specific IgG is detected with the aid of a second antibody known as an antiglobulin. Antiglobulins are essential reagents in many serological tests and are prepared by immunizing another host (e.g., rabbit) with IgG isolated from the same species of animal (e.g., mule deer) as the one in which diagnosis is to be attempted. Conjugation of the antiglobulins to an indicator, e.g., an enzyme, provides an excellent way of monitoring the binding of antiglobulins and the assessment of a serological test. An enzyme-conjugated antiglobulin is called an antibody conjugate. An example of an antibody conjugate is rabbit antiglobulin produced following immunization with mule deer IgG, and subsequently conjugated to alkaline phosphatase, i.e., alkaline phosphatase (AP) conjugated anti-mule deer IgG.

Antibody conjugate for one specialized livestock species, the WTD, is available on the market and retails for \$81.49 per 100 µg vial (Kirkegaard and Perry Laboratories, Gaithersburg, Maryland, USA). Antibody conjugates for other species (bison, fallow deer, mule deer, llama, alpacas, emu or ostriches) are not available commercially or otherwise.

It is therefore necessary and urgent to develop antibody conjugates that are not available on the market, and which can be used for the quick and reliable serological diagnosis of infectious diseases of specialized livestock. Once produced, the antibody conjugates could be produced in commercial quantities to facilitate the development of new diagnostic tests.

The objective of the project is to produce high quality antibody conjugates which can be used for the serological diagnosis of diseases of the following specialized livestock species: bison, caribou and mule deer.

PROJECT ACTIVITIES

Blood collection and isolation of IgG

Blood samples were collected from bison, caribou and mule deer and serum fractions obtained. Immunoglobulin class G (IgG) antibodies were obtained from each species by passing serum over a column (Protein G column) to trap the IgG which on release was further purified by a chromatographic procedure (anion-exchange for bison IgG or gel filtration for caribou and mule deer IgG).

Immunization of rabbits

Purified bison, caribou, and mule deer IgG were mixed separately with an adjuvant, Complete Freund's Adjuvant (CFA), and each injected into four rabbits, subcutaneously. Two weeks later, a booster immunization of the IgG preparations mixed this time, with Incomplete Freund's Adjuvant was administered to the rabbits. Once peak antibody production were attained, rabbits were bled out and sera collected.

Affinity purification of antibody conjugates

Anti-globulins (anti-bison, -caribou, -mule deer) were purified from immunized rabbit sera with the aid of Protein G sepharose. Purified antiglobulins were assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and found to be very pure.

Conjugation of enzyme to antiglobulins to produce antibody conjugates and lyophilization

Two enzymes were used for conjugation, namely alkaline phosphatase (AP) and horse-radish peroxidase (HRP). The quantity and quality of antibody conjugates produced were assessed by determining the protein concentration and by ELISA test to determine the working dilutions of the conjugates in ELISA.

	Anti-bison		Anti-caribou		Anti-mule deer	
	HRP	AP	HRP	AP	HRP	AP
Total protein concentration (mg)	3.9	3.7	10.6	4.1	7.6	3.8
ELISA working dilution						
(Reciprocal of dilution)	1,000	500	4,000	500	4,000	4,000

The newly produced bison, caribou, and mule deer conjugates are of similar (2 of 6) or higher quality (4 of 6) than the commercially available WTD conjugate, which is routinely used at a dilution of 1:500.

Papers written

None.

Results and Conclusions

High quality antibody conjugates for serological diagnosis of diseases of bison, caribou, and mule deer have been produced. The antibodies were conjugated to either of two enzymes thus providing a choice for prospective users.

Information on performance indicators

New products for a better health management of bison, caribou and mule deer have been produced. Rapid diagnoses of infectious diseases of these species should now be possible.

Indications for further development

The production of conjugates for other specialized livestock species such as fallow deer, llama, alpacas, emu and ostriches will aid in the diagnoses of diseases of these species and is hereby recommended.

PERSONNEL

The following personnel were involved in the project from July 20, 1998 to February 1, 2000.

<u>Name</u>	<u>Amount of time</u>	<u>Salary (\$)</u>
Shaun Dergousoff	66%	32,347

EQUIPMENT

No equipment was purchased or rented in the reporting period.

PROJECT PHOTOS

Not applicable.

INNOVATION FUND ACKNOWLEDGEMENT

AFIF support was acknowledged at the following presentations:

Meeting between members of Centre of Animal Parasitology (CAP) and the Board of Directors of AFIF on January 28, 1999 at the Health of Animals Laboratory, Saskatoon.

Seminar presented to faculty and students of the Department of Veterinary Microbiology, University of Saskatchewan, Saskatoon on February 22, 1999.

PROJECT PUBLIC INFORMATION MATERIALS

Plans are underway to commercialize the newly-produced antibody conjugates. It is envisaged that diagnosticians and other relevant discipline groups will be informed of the availability of the conjugates through relevant discussion groups on the Internet (e.g., vetImm, deerNet) once a company willing to market the products has been found.

EXPENSE STATEMENT

A final expense statement is attached.